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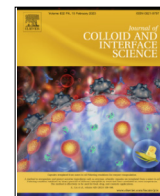
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# Shape-Shifting Thermoresponsive Block Copolymer Nano-Objects

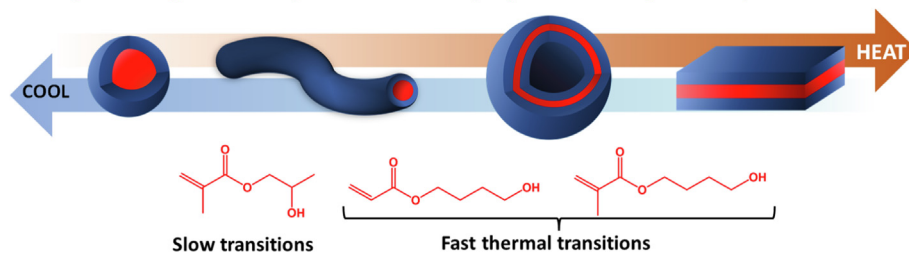
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## GRAPHICAL ABSTRACT

This Feature Article focuses on the rational design of 'shape-shifting' thermoresponsive diblock copolymer nano-objects prepared using 2 hydroxypropyl methacrylate, 4 hydroxybutyl acrylate or hydroxybutyl methacrylate. A subtle change in the *partial* degree of hydration of the permanently insoluble thermoresponsive block drives thermal transitions between spheres, worms, vesicles and lamellae. Potential applications for this fascinating new class of amphiphiles are suggested.

### Shape-shifting thermoresponsive diblock copolymer nano-objects in aqueous solution



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## ABSTRACT

In this *Feature Article*, we review our recent progress in the design of shape-shifting thermoresponsive diblock copolymer nano-objects, which are prepared using various hydroxyl-functional (meth)acrylate monomers (e.g. 2 hydroxypropyl methacrylate, 4 hydroxybutyl acrylate or hydroxybutyl methacrylate) to generate the thermoresponsive block. Unlike traditional thermoresponsive polymers such as poly(*N*-isopropylacrylamide), there is no transition between soluble and insoluble polymer chains in aqueous solution. Instead, thermally driven transitions between a series of copolymer morphologies (e.g. spheres, worms, vesicles or lamellae) occur on adjusting the aqueous solution temperature owing to a subtle change in the *partial* degree of hydration of the permanently insoluble thermoresponsive block. Such remarkable self-assembly behavior is unprecedented in colloid science: no other amphiphilic diblock copolymer or surfactant system undergoes such behavior at a fixed chemical composition and concentration. Such shape-shifting nano-objects are characterized by transmission electron microscopy, dynamic light scattering, small-angle X-ray scattering, rheology and variable temperature <sup>1</sup>H NMR spectroscopy. Potential applications for this fascinating new class of amphiphiles are briefly considered.

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## 1. Introduction

It is well known that many non-ionic water-soluble polymers exhibit inverse temperature solubility behavior in aqueous solution.[1–10] Perhaps the most common example in the literature is poly(*N*-isopropylacrylamide), which is soluble in cold water

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but becomes insoluble in hot water.[1–4] In this particular case, there is a relatively well-defined cloud point of approximately 32 °C, which is accompanied by a coil-to-globule transition (see Fig. 1).[5,6] Various other water-soluble polymers exhibit rather less well-defined ('smeared out') transitions.[7–10].

We emphasize that this *Feature Article* is not concerned with such thermoresponsive behavior. Instead, we focus on a new class of so-called shape-shifting thermoresponsive diblock copolymer nano-objects, which can be prepared using various hydroxyl-functional (meth)acrylic monomers (e.g. 2-hydroxypropyl methacrylate, 4-hydroxybutyl acrylate or hydroxybutyl methacrylate) to generate the thermoresponsive block. Raising the solution temperature of aqueous dispersions of such diblock copolymer nano-objects leads to a subtle change in the *partial* degree of hydration of the permanently insoluble thermoresponsive block, which in turn drives thermal transitions between the four most common copolymer morphologies (e.g. spheres, worms, vesicles or lamellae), see Fig. 2.

The field of block copolymer nanoparticles has evolved substantially over the past six decades.[11–23] Traditionally, well-defined diblock copolymers were prepared in the form of molecularly-dissolved chains via synthetically demanding techniques such as anionic polymerization [24–26] and then self-assembled in dilute

solution using post-polymerization processing techniques such as solvent exchange, a pH switch or thin film rehydration.[11,27–32] However, the development of living radical polymerization techniques such as reversible addition–fragmentation chain transfer (RAFT) polymerization has enabled the straightforward synthesis of many new examples of functional amphiphilic block copolymers.[33–42] Moreover, remarkable progress in the field of polymerization-induced self-assembly (PISA) over the past decade or so has led to many literature examples of the efficient synthesis of diblock copolymer nano-objects directly in solution.[15,17,21–23,43,44] Importantly, the radical nature of RAFT chemistry enables the direct polymerization of a wide range of *functional* vinyl monomers, such as the three hydroxyl-functional (meth)acrylic monomers that are the focus of this *Feature Article* (see Fig. 3).[45–64]

PISA has been used to prepare many examples of amphiphilic diblock copolymers in the form of sterically-stabilized nanoparticles in aqueous solution.[51,65–84] This can be achieved by either RAFT aqueous emulsion polymerization or RAFT aqueous dispersion polymerization. The former process involves water-immiscible monomers such as styrene,[73,74,77,85–87] methyl methacrylate,[72,80] benzyl methacrylate [88,89] *n*-butyl acrylate [80,86,90] phenyl acrylate,[91] or 2,2,2-trifluoroethyl methacrylate.[92] These monomers produce relatively hydrophobic blocks that cannot exhibit thermoresponsive behavior. By definition, the latter process is confined to water-miscible monomers such as 2-hydroxypropyl methacrylate (HPMA) or 4-hydroxybutyl acrylate (HBA) (see Fig. 3). Polymerization of such monomers leads to weakly hydrophobic blocks that can exhibit thermoresponsive behavior. For example, in 2012 we reported that the RAFT aqueous dispersion polymerization of HPMA using a water-soluble poly(glycerol monomethacrylate) (PGMA) precursor leads to the formation of PGMA<sub>54</sub>-PHPMA<sub>140</sub> worms,[47] which form soft gels at 20 °C in semi-concentrated aqueous solution owing to the formation of a 3D percolating network.[81] On cooling to 4–5 °C, these worms are transformed into spheres, which leads to *in situ* degelation. This morphological transition is fully thermoreversible, as judged by dynamic light scattering (DLS), transmission electron

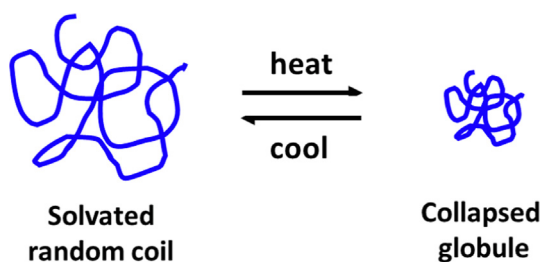


Fig. 1. Schematic cartoon of the thermally-induced coil-to-globule transition exhibited by a non-ionic water-soluble polymer such as poly(*N*-isopropylacrylamide) (PNIPAM) in aqueous solution.[5,6]

### Shape-shifting thermoresponsive AB diblock copolymer nano-objects

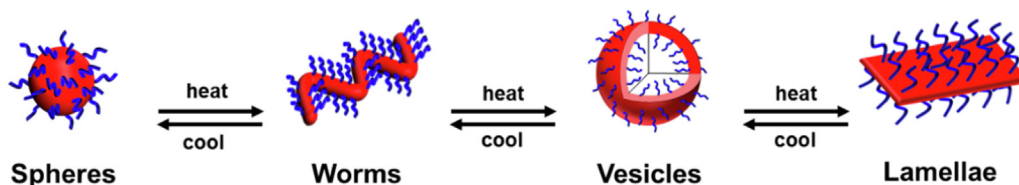


Fig. 2. Shape-shifting thermoresponsive behavior exhibited by the amphiphilic diblock copolymers highlighted in this *Feature Article*, which can form spheres, worms, vesicles or lamellae reversibly in aqueous solution on varying the solution temperature.

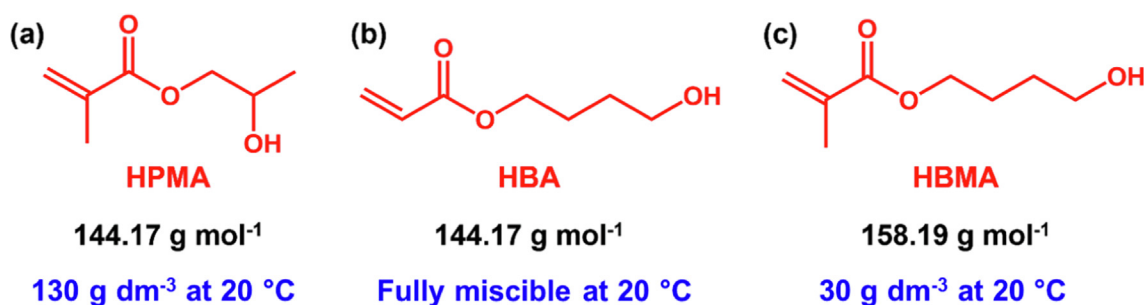


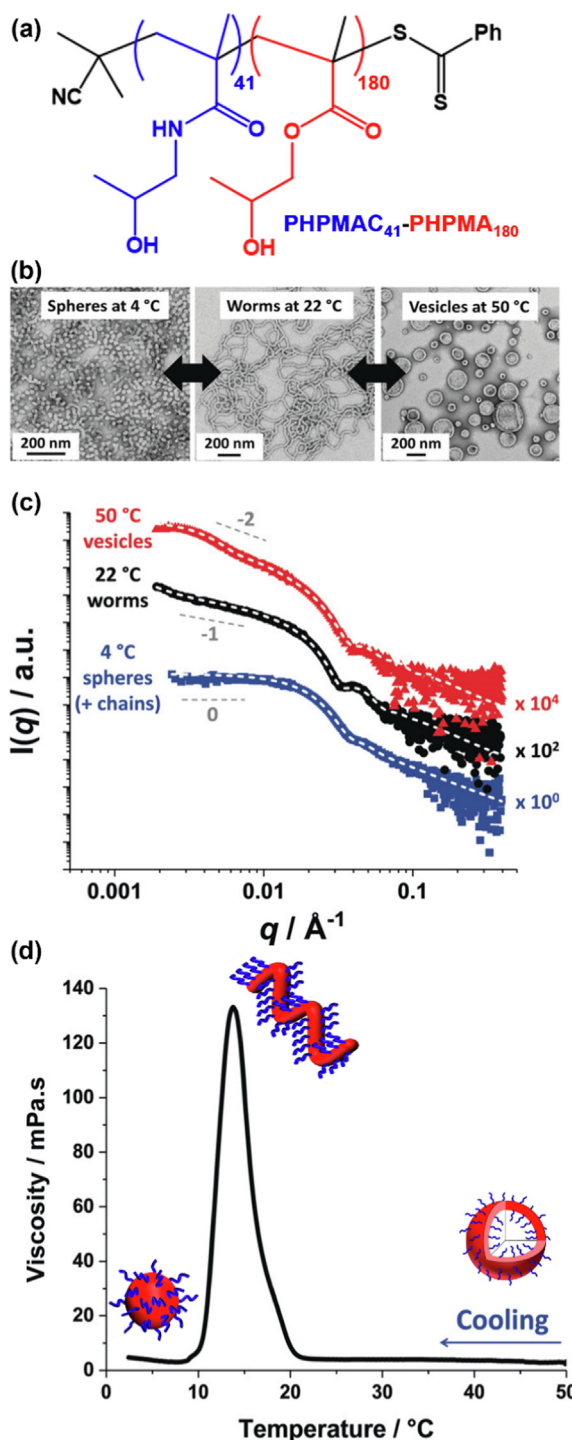
Fig. 3. Chemical structures, molecular weights, and aqueous solubilities at 20 °C for: (a) 2-hydroxypropyl methacrylate (HPMA; only the major isomer is shown in this case); (b) 4-hydroxybutyl acrylate (HBA); (c) hydroxybutyl methacrylate (HBMA; only the 4-isomer is shown in this case).

microscopy (TEM), small-angle X-ray scattering (SAXS) and rheological studies. Such thermoresponsive worm gels are highly biocompatible and have been evaluated for (i) the long-term storage of human stem cells,[93] (ii) the preservation of red blood cells,[94] and (iii) as an alternative 3D cell culture medium to *Matrigel*.[95] Similar behavior has been observed for HPMA-based worm gels prepared using alternative water-soluble blocks such as poly(ethylene glycol) (PEG)[51,96–99] or poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC).[99–101] Interestingly, the thermoresponsive behavior of the PHPMA block depends on its *absolute* degree of polymerization (DP): if the PHPMA DP is too long, such chains become permanently hydrophobic.[51,82,98] This constraint places an upper limit on the mean cross-sectional diameter for thermoresponsive PHPMA-based worms. In related work, Warren et al. reported that PEG<sub>113</sub>-PHPMA<sub>300</sub> vesicles exhibited a vesicle-to-sphere transition on cooling from 25 °C to 2 °C.[51] Subsequently, Mable and co-workers reported similar thermoresponsive behavior for PGMA<sub>59</sub>-PHPMA<sub>250</sub> vesicles, which was exploited for the thermally-triggered release of silica nanoparticles that had been encapsulated during the PISA synthesis of such nano-objects.[53,102]

In 2019, we reported that using poly(2 hydroxypropyl methacrylamide) (PHPMAC) as a precursor for the RAFT aqueous dispersion polymerization of HPMA leads to the formation of a well-defined PHPMAC<sub>41</sub>-PHPMA<sub>180</sub> diblock copolymer.[55] This thermoresponsive copolymer was the first example of an amphiphile that could form the three main morphologies (spheres, worms or vesicles) reversibly in aqueous solution simply by adjusting the solution temperature, as confirmed by variable temperature TEM and SAXS studies (see Fig. 4).[103]

Given the remarkably similar chemical structures for the hydrophilic PHPMAC and hydrophobic PHPMA blocks (their repeat units differ by just 1 g mol<sup>-1</sup>), this system is particularly well-suited to analysis using mean field theory. Indeed, our collaborators Prof. Remco Tuinier and Dr. Alessandro Ianiro at the Technical University of Eindhoven were able to account for both the sphere-to-worm and worm-to-vesicle transitions by making a judicious estimate of the associated Flory-Huggins parameter (e.g.  $\chi_{\text{HPMA-W}}$ ) for this system, see Fig. 5.[55] However, this PHPMAC<sub>41</sub>-PHPMA<sub>180</sub> exhibited strong hysteresis: under certain conditions, the observed morphological transitions can be extremely slow. For example, acquisition of the rheological data shown in Fig. 4d required a cooling rate of just 0.5 °C per hour. This problem is attributed to the relatively low mobility of the PHPMA chains, which exhibit a glass transition temperature ( $T_g$ ) of around 90–95 °C.[46,58] Given this limitation, this particular diblock copolymer is perhaps best regarded as a proof-of-concept prototype.

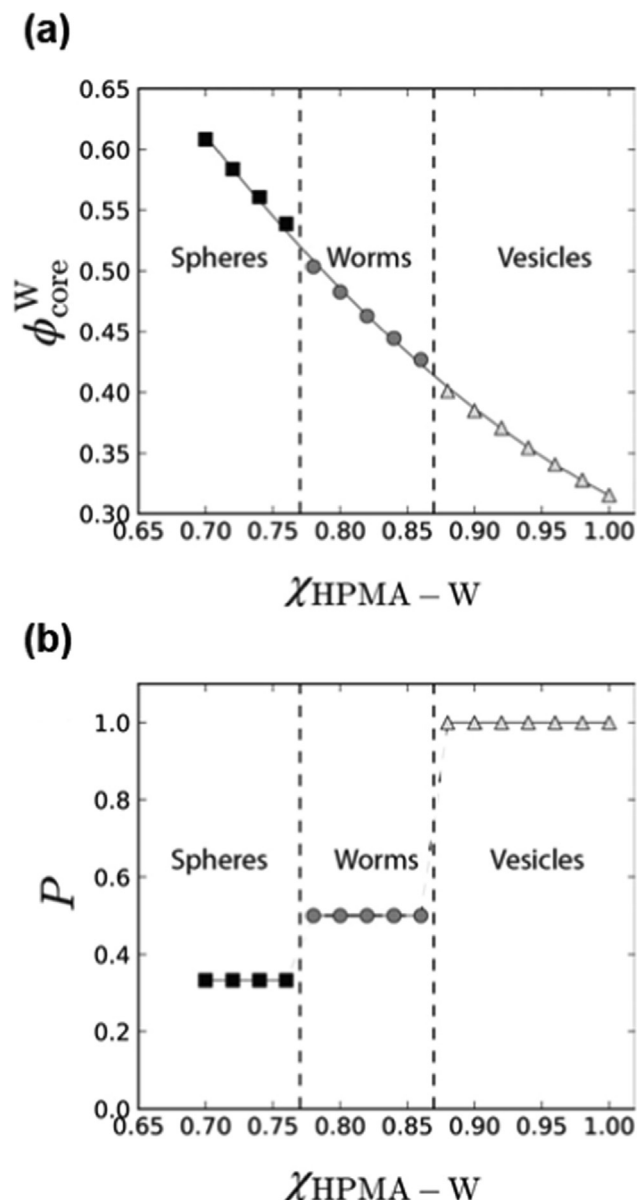
To address this chain mobility problem, we identified HBA (see Fig. 3) as a potentially interesting alternative monomer for the construction of the weakly hydrophobic block. This monomer is fully water-miscible in all proportions, whereas HPMA has an aqueous solubility of approximately 13 % w/w at 20 °C.[104] This difference immediately suggests that PHBA (which is water-insoluble) should be more weakly hydrophobic than PHPMA. Moreover, the former acrylic homopolymer should exhibit a significantly lower  $T_g$  than the latter methacrylic homopolymer. This hypothesis was supported by the striking difference in their physical appearance: PHBA is a transparent sticky gum whereas PHPMA is a white free-flowing powder. Accordingly, we examined the RAFT aqueous dispersion polymerization of HBA using poly(*N,N'*-dimethylacrylamide) (PDMAC) as a water-soluble precursor and (DDMAT) as the RAFT agent.[105] Preliminary characterization using DLS, SAXS and rheological measurements suggested that the resulting PDMAC-PHBA nano-objects were thermoresponsive. However, the sub-ambient  $T_g$  of the PHBA block prevented TEM studies, so the predominant copolymer morphology after drying



**Fig. 4.** (a) Synthesis of PHPMAC<sub>41</sub>-PHPMA<sub>180</sub> via RAFT aqueous dispersion polymerization of HPMA at 70 °C. This was the first example of a block copolymer that could form three different morphologies reversibly in aqueous solution simply by adjusting the solution temperature. More specifically, (b) TEM and (c) SAXS studies showed that this PHPMAC<sub>41</sub>-PHPMA<sub>180</sub> block copolymer forms spheres at 4 °C, worms at 22 °C, and vesicles on heating to 50 °C. However, the viscosity vs temperature plot shown in (d) for a 10 % w/w aqueous dispersion of the PHPMAC<sub>41</sub>-PHPMA<sub>180</sub> block copolymer nano-objects required a very slow cooling rate (just 0.5 °C h<sup>-1</sup>) owing to the strong hysteresis exhibited by the copolymer chains when switching between morphological transitions.[55]

at any given temperature could not be confirmed. Moreover, our concerted attempts to conduct cryo-TEM studies on such nano-objects were unsuccessful. To circumvent this problem, we statis-

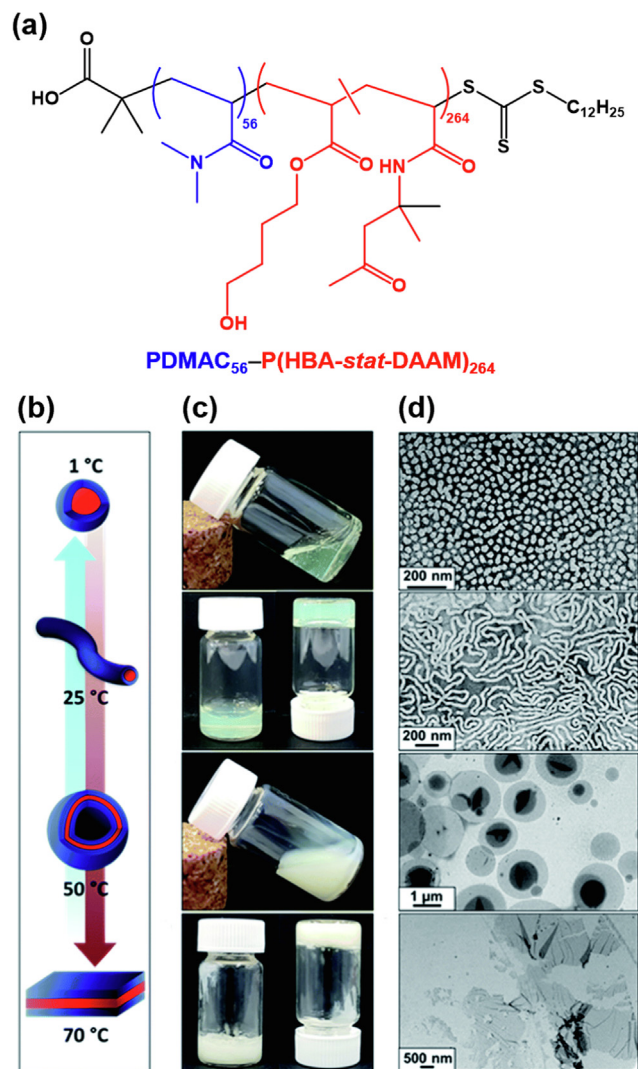




**Fig. 5.** (a) Variation in the mean volume fraction of water associated with the hydrophobic core-forming PHPMA block  $\phi_{\text{core}}^W$  and (b) the molecular packing parameter  $P$  as a function of  $\chi_{\text{HPMA-W}}$ , as calculated for  $\text{PHPMAC}_{41}\text{-PHPMA}_{180}$ . The vertical dashed lines denote the sphere/worm and worm/vesicle boundaries.[55]

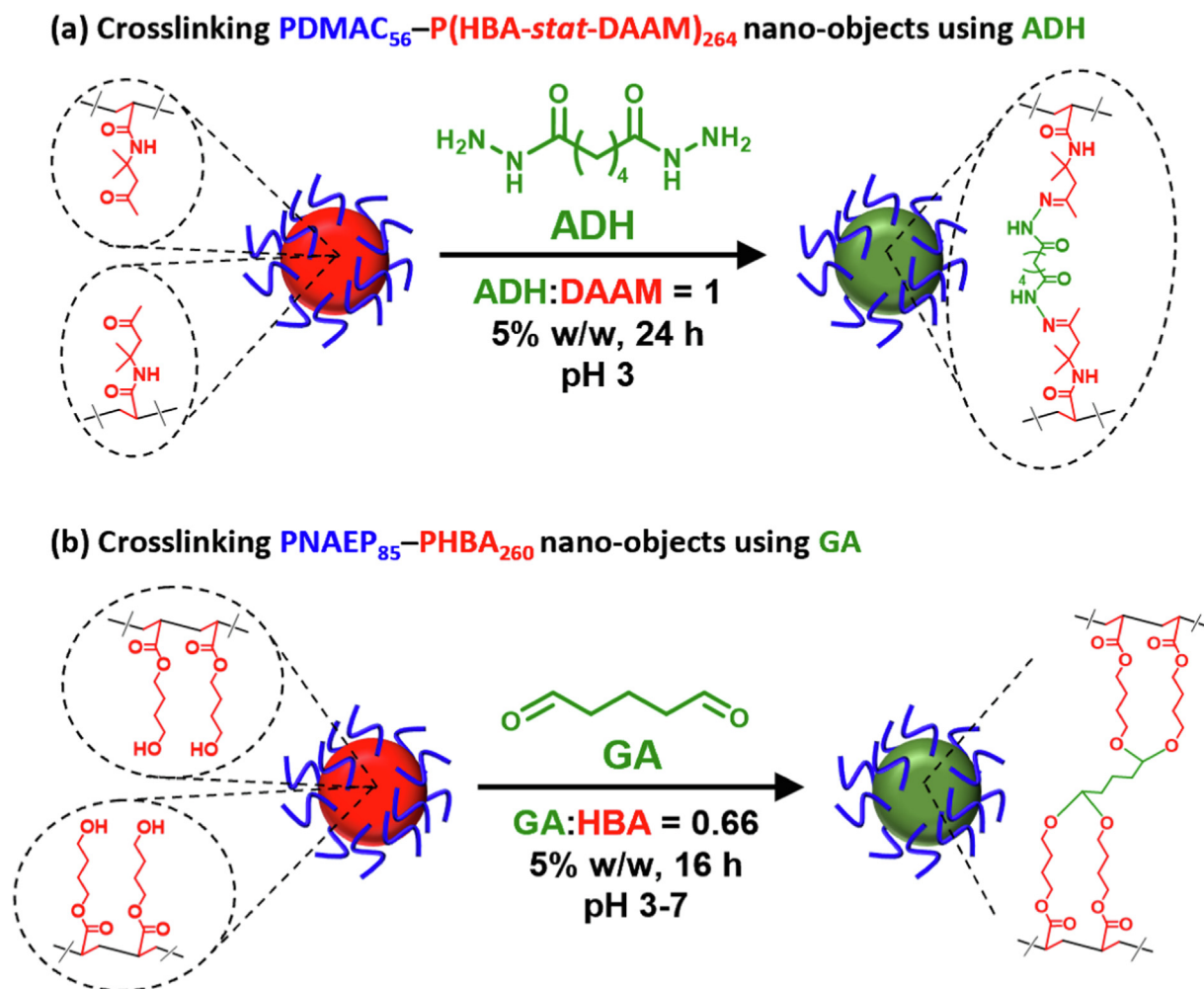
tically copolymerized 20 mol% diacetone acrylamide (DAAM) with 80 mol% HBA, see Fig. 6[56] DAAM is a water-miscible comonomer bearing a pendent methyl ketone group: this enables the resulting hydrophobic P(HBA-*stat*-DAAM) chains to be crosslinked using adipic acid dihydrazide (ADH), as depicted in Fig. 7a.[56,106,107]

Such covalent stabilization enabled the copolymer morphology to be ‘fixed’ at a given aqueous solution temperature while also raising the  $T_g$  of the hydrophobic chains sufficiently to allow meaningful TEM studies (see Fig. 6). Thus it was confirmed that  $\text{PDMAC}_{56}\text{-P(HBA-}i\text{stat-DAAM)}_{264}$  forms spheres at 1 °C, worms at 25 °C, vesicles at 50 °C and lamellae at 70 °C. This was the first time that four distinct morphologies had been reported for a single diblock copolymer of fixed composition at a constant concentration. The thermoresponsive behavior of the linear copolymer was characterized by rheology, SAXS and DLS at copolymer concentrations of 10 % w/w, 1.0 % w/w and 0.1 % w/w, respectively. Each technique indicated the formation of spheres, worms and vesicles



**Fig. 6.** (a) Chemical structure of the  $\text{PDMAC}_{56}\text{-P(HBA-}i\text{stat-DAAM)}_{264}$  diblock copolymer. (b) Schematic representation of the reversible morphological transitions that occur for a 20 % w/w aqueous dispersion of  $\text{PDMAC}_{56}\text{-P(HBA-}i\text{stat-DAAM)}_{264}$  nano-objects on varying the temperature from 1 °C to 70 °C. Digital images show the physical appearance of this aqueous dispersion: (b) on cooling to 1 °C for 30 min, (c) at ambient temperature (25 °C), (d) on heating to 50 °C for 30 min and (e) on heating to 70 °C for 30 min. (c) TEM images recorded for 0.10 % w/w aqueous dispersions of  $\text{PDMAC}_{56}\text{-P(HBA-}i\text{stat-DAAM)}_{264}$  nano-objects: spheres (crosslinked at 1 °C), worms (crosslinked at 25 °C), vesicles (crosslinked at 50 °C) and lamellae (crosslinked at 70 °C). [N.B. Uranyl formate was employed as a negative stain after covalent stabilization of each nano-object at the desired temperature using adipic acid dihydrazide (ADH) at a DAAM/ADH molar ratio of 1.0]. [56]

with good thermoreversibility. Shear-induced polarized light imaging (SIPLI) was used to confirm the presence of lamellae and significant hysteresis was observed for the vesicle-to-lamellae transition. Variable temperature  $^1\text{H}$  NMR studies indicated a monotonic increase in the degree of partial hydration of the weakly hydrophobic P(HBA-*stat*-DAAM) chains at higher temperature, suggesting that each morphological transition is driven by *uniform* plasticization, rather than *surface* plasticization.[108] In essence, this means that the entire length of the P(HBA-*stat*-DAAM) chains becomes slightly more hydrated, rather than just those repeat units located nearest to the block junction (see Fig. 8). This is termed a ‘UCST-like’ transition because it involves greater (albeit partial) hydration of the weakly hydrophobic block at *higher* temperature. Such behavior differs qualitatively from that observed for PHPMA-based thermoresponsive diblock copolymers,



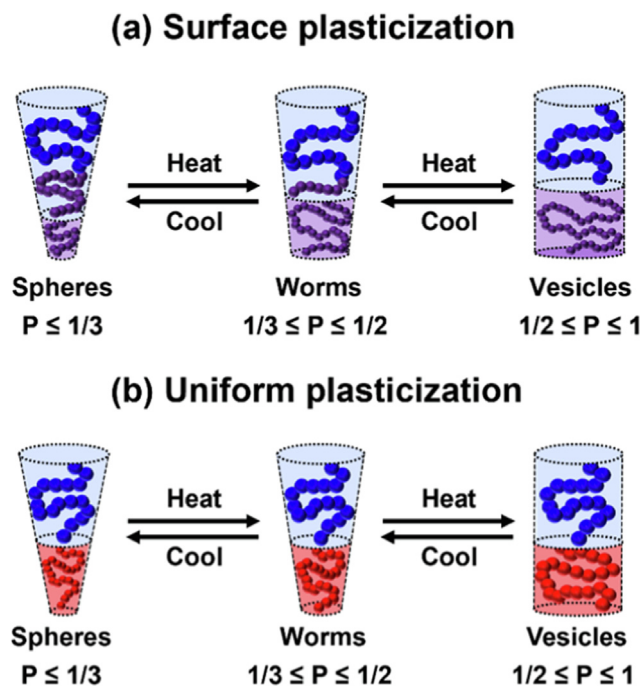
**Fig. 7.** Schematic cartoon illustrating the intermolecular crosslinking achieved (a) for the  $\text{PDMAC}_{56}\text{-P(HBA-}i\text{stat-DAAM)}_{264}$  nano-objects via reaction of the pendent ketone groups on the DAAM repeat units with adipic acid dihydrazide (ADH) at a DAAM/ADH molar ratio of 1.0 or (b) for the PHBA chains within  $\text{PNAEP}_{85}\text{-PHBA}_{260}$  nano-objects by reaction of the pendent hydroxyl groups with excess glutaraldehyde (GA).[56,57]

which is best explained in terms of *surface plasticization*.<sup>[17]</sup> Thus, HPMA repeat units located near the block junction become hydrated, which leads to an effective increase in the volume fraction occupied by the stabilizer block: this is sufficient to lower the fractional packing parameter,  $P$ ,<sup>[109,110]</sup> and hence drive the morphological evolution from vesicles to worms to spheres. We denote this as an ‘LCST-like’ transition because greater (albeit partial) hydration of the weakly hydrophobic block occurs at *lower* temperature.

In retrospect, one drawback of this study was the use of a carboxylic acid-based RAFT agent for the synthesis of the  $\text{PDMAC-P(HBA-}i\text{stat-DAAM)}$  diblock copolymer. This means that the shape-shifting thermoresponsive behavior can only be observed at low pH, which suppresses ionization of this end-group. In contrast, the anionic carboxylate groups that are formed at neutral pH led to the formation of kinetically-trapped spheres, with Lovett and co-workers reporting similar observations for the  $\text{PGMA-}i\text{PHMA}$  system.<sup>[54]</sup> In principle, this limitation can be eliminated by using the methyl ester analog of the RAFT agent and conducting an initial bulk polymerization of DMAC to ensure solubility of this more hydrophobic RAFT agent.

Indeed, this subtle but important refinement was employed when we recently revisited this  $\text{PDMAC}_n\text{-P(HBA-}i\text{stat-DAAM)}_m$  formulation to explore whether such a shape-shifting thermoresponsive diblock copolymer might enable a microencapsulation

strategy to be devised at neutral pH.<sup>[111]</sup> Thus,  $\text{PDMAC}_{49}\text{-P(HBA-}i\text{stat-DAAM)}_{302}$  was prepared as a freeze-dried powder and added to cold water at 1 °C. An aqueous dispersion of 20 nm silica nanoparticles (also cooled to 1 °C) was added to afford a binary mixture of organic and inorganic nanoparticles, which was then heated to 50 °C. This led to the formation of  $\text{PDMAC}_{49}\text{-P(HBA-}i\text{stat-DAAM)}_{302}$  vesicles with the concomitant encapsulation of some of the silica nanoparticles, see Fig. 9. A series of systematic studies were undertaken in which (i) the silica nanoparticle concentration was varied at a fixed copolymer concentration and (ii) the copolymer concentration was varied at a fixed silica nanoparticle concentration. The loading efficiency of the silica nanoparticles within the vesicles could be conveniently evaluated by thermogravimetry because the copolymer is fully pyrolyzed above 650 °C whereas the silica nanoparticles remain thermally stable. The highest loading efficiency was 86 %, which was achieved at an initial silica nanoparticle of 2.5 % w/w (see Fig. 10a). One novel aspect of this study was the use of a disulfide-based adipic acid dihydrazide crosslinker (DS-ADH). This enabled preservation of the vesicle morphology - and hence retention of the encapsulated silica nanoparticle cargo - at any desired temperature while allowing triggered release on demand via selective cleavage of the disulfide bonds via redox chemistry using tris(2-carboxyethyl)phosphine (TCEP). This was confirmed by TEM analysis before and after TCEP addition (see Fig. 10b-c), which indicated the presence of silica-

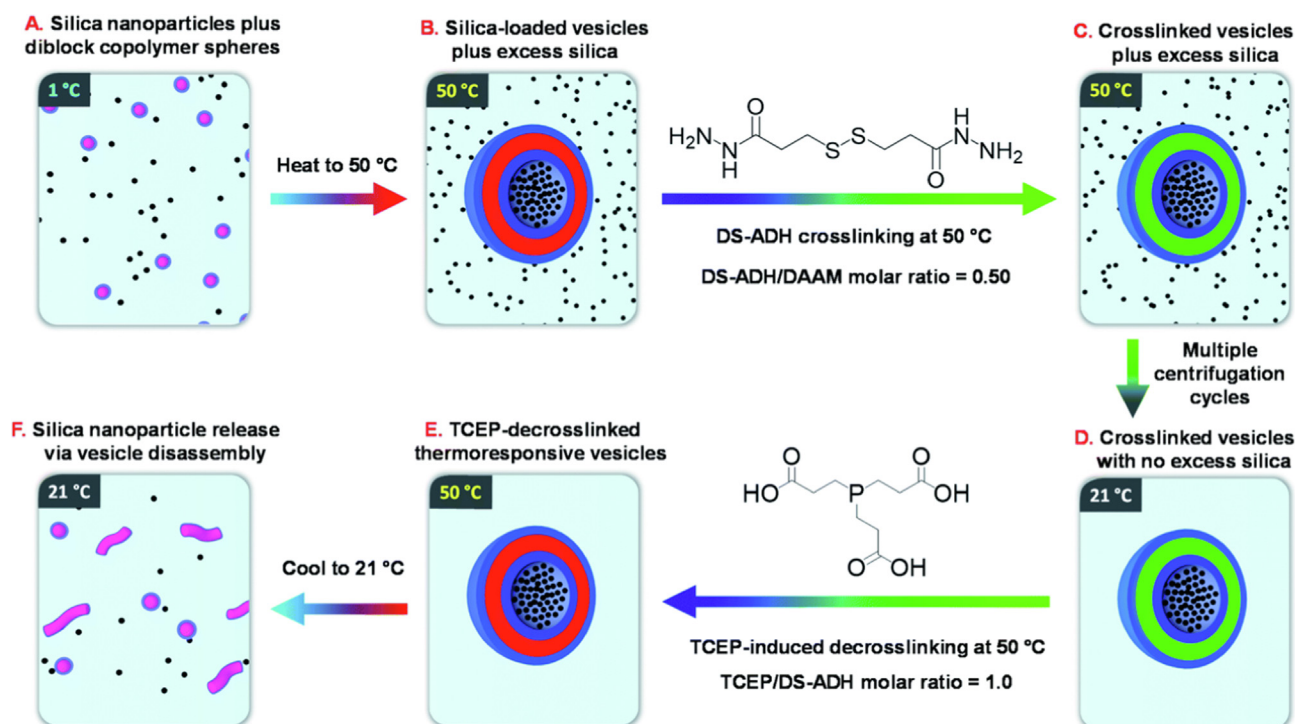


**Fig. 8.** Schematic cartoons indicating (a) the surface plasticization exhibited by thermoresponsive **PGMA-PPHMA** and **PEG-PPHMA** nano-objects (note the subtle shift in the effective block junction) and (b) the uniform plasticization exhibited by thermoresponsive **PHBA**-based nano-objects. The associated copolymer morphologies and their characteristic packing parameters are also indicated.<sup>[58]</sup>

loaded vesicles and free silica nanoparticles, respectively. Overall, these results augur well for the efficient encapsulation of enzymes within such vesicles for the design of new protocells and model

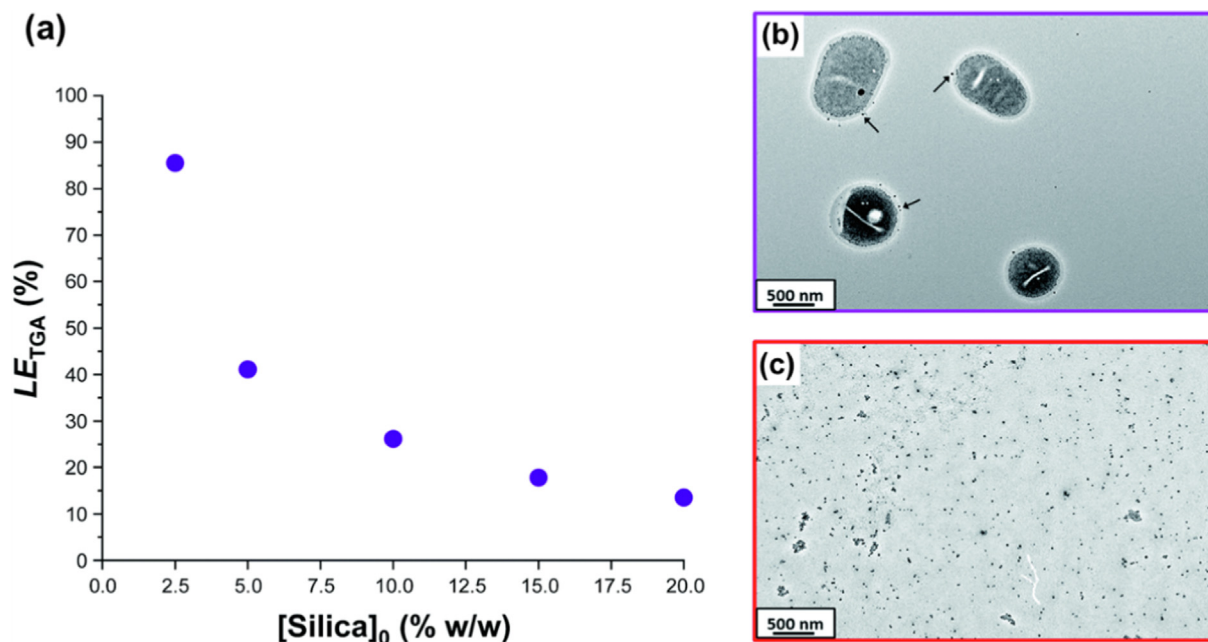
cascade reaction systems. We intend to explore these possibilities in future work.

Unfortunately, statistical copolymerization of DAAM with HBA to enable covalent stabilization of each copolymer morphology tends to reduce the desired thermoresponsive behavior. To address this issue, we designed a new all-acrylic diblock copolymer system, see Fig. 11a.<sup>[57]</sup> A carboxylic acid-functionalized RAFT agent was employed to polymerize 2-*N*-(acryloyloxy)ethyl pyrrolidone (NAEP) in aqueous media using a low temperature redox initiator at pH 3. Once this polymerization was complete, the water-soluble PNAEP precursor was chain-extended with HBA to produce an amphiphilic PNAEP-PHBA diblock copolymer, which typically self-assembles to form vesicles at the reaction temperature of 30 °C. Such linear copolymer nano-objects cannot be visualized by TEM owing to film formation during grid preparation. However, crosslinking can be achieved in acidic aqueous media using glutaraldehyde, which reacts with the pendent hydroxy groups on the PHBA chains, (see Fig. 7b). Such covalent stabilization is sufficient to enable confirmation of the formation of spheres, worms, vesicles and lamellae by a PNAEP<sub>85</sub>-PHBA<sub>295</sub> diblock copolymer on varying the solution temperature. Subsequent rheology, DLS and SAXS studies of the behavior of the linear PNAEP<sub>85</sub>-PHBA<sub>295</sub> nano-objects confirmed excellent reversibility for the sphere/worm and worm/vesicle morphological transitions (see Fig. 11). However, significant hysteresis was again observed for the vesicle/lamellae transition, just like the earlier PDMAC<sub>49</sub>-P(HBA-*stat*-DAAM)<sub>302</sub> system. This suggests that this may be an intrinsic feature of this morphological transition, rather than a specific problem associated with a particular diblock copolymer system. Indeed, it seems likely that such hysteretic behavior is related to the initial bending and eventual wrap up of the lamellae to form vesicles, for which there is an associated energy penalty. Moreover, the lamellae must also undergo fragmentation, because the mean



**Fig. 9.** Schematic cartoon of the six stages involved in the encapsulation and release of silica nanoparticles using a thermoresponsive shape-shifting **PDMAc<sub>49</sub>-P(HBA-*stat*-DAAM)<sub>302</sub>** diblock copolymer that forms spheres at 1 °C and vesicles at 50 °C. The linear vesicles are crosslinked using DS-ADH while subsequent decrosslinking is achieved on demand via redox chemistry using TCEP.<sup>[111]</sup>





**Fig. 10.** (a) Vesicle loading efficiency,  $LE_{TGA}$ , as determined by thermogravimetric analysis for a series of  $PDMAC_{49}$ - $P(HBA\text{-}stat\text{-}DAAM)_{302}$  vesicles prepared at various silica nanoparticle concentrations using a constant copolymer concentration of 10% w/w. Representative TEM images recorded (b) before and (c) after TCEP addition confirm the presence of silica-loaded vesicles and free silica nanoparticles, respectively.[111]

aggregation number of the vesicles is significantly lower than that of the lamellae.

Importantly, systematic variation of the PHBA DP from 110 to 475 when using a  $PNAEP_{85}$  stabilizer block enabled the critical temperature for the pure worm phase (which corresponds to the maximum viscosity in a viscosity vs temperature plot) to be varied from around 0 °C up to approximately 50 °C (see Fig. 12). In this context, it is perhaps worth emphasizing that the highest PHBA DP of 475 still produced thermoresponsive nano-objects, whereas the equivalent PHPMA block would certainly remain permanently hydrophobic (i.e., non-thermoresponsive).[112].

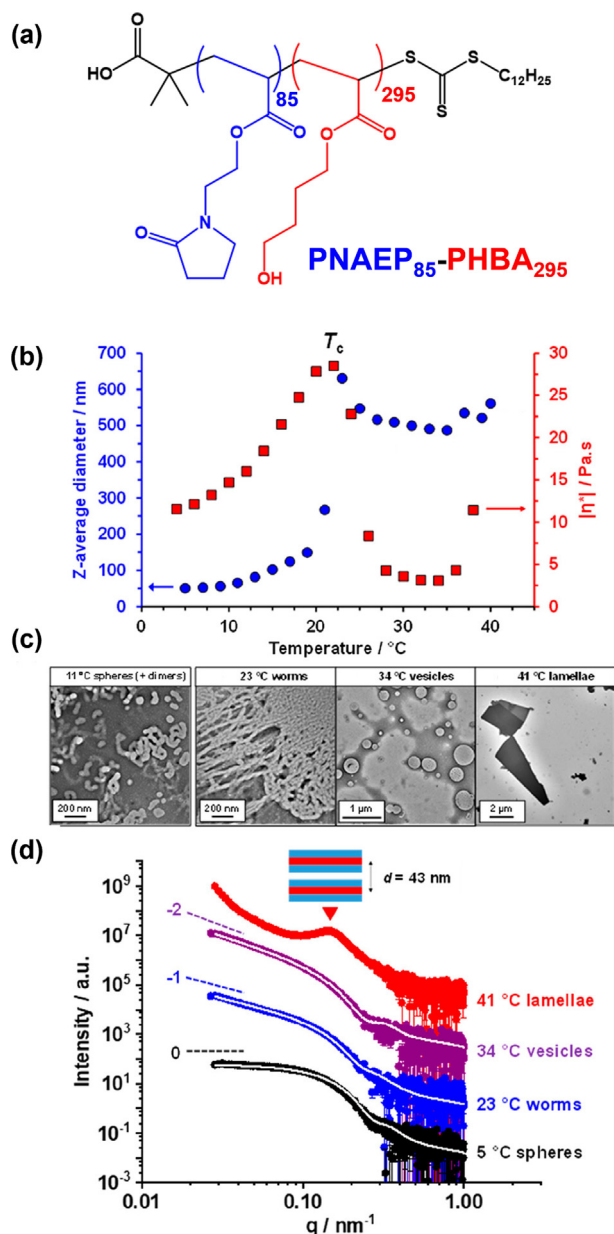
Like the  $PDMAC$ - $P(HBA\text{-}stat\text{-}DAAM)$  diblock copolymer formulation discussed above, this  $PNAEP$ - $PHBA$  system only exhibits shape-shifting thermoresponsive behavior in weakly acidic aqueous solution (pH 3). At higher pH, ionization of the terminal carboxylic acid group located on the end of the  $PNAEP$  stabilizer chains occurs, which results in the formation of kinetically-trapped anionic spheres. Again, this problem can be addressed simply by choosing the methyl ester analog of the trithiocarbonate-based RAFT agent used for the  $PNAEP$ - $PHBA$  synthesis, although this might require further optimization of the formulation (e.g. a higher initial  $NAEP$  concentration) to ensure its solubility in the aqueous milieu. This synthetic refinement should ensure that the desired shape-shifting behavior is observed at neutral pH, which would be beneficial for potential bio-applications.

In a follow-up study, Deane and co-workers prepared a series of  $PHBA$ -based diblock copolymers using a poly(ethylene glycol) (PEG) water-soluble precursor (see Fig. 13a).[58] It is perhaps worth emphasizing that this approach eliminates the problem of end-group ionization and allows the desired shape-shifting thermoresponsive behavior to be observed at neutral pH. A phase diagram was constructed by systematically varying the target  $PHBA$  DP and the solution temperature (see Fig. 13b). This enabled identification of a suitable diblock copolymer composition ( $PEG_{113}$ - $PHBA_{350}$ ) that formed spheres, worms, vesicles or lamellae within a relatively narrow temperature range (6–50 °C).

Such morphological assignments were initially made on the basis of TEM analysis - after covalent stabilization of each type of nano-object using glutaraldehyde - and subsequently confirmed by SAXS studies. Importantly, the choice of PEG as the steric stabilizer enabled all the proton signals assigned to the  $PHBA$  block to be observed, which facilitated variable temperature  $^1H$  NMR experiments. In this case, the partial degree of hydration of a  $PEG_{113}$ - $PHBA_{260}$  diblock copolymer was compared to that of a  $PEG_{113}$ - $PHPMA_{260}$  diblock copolymer prepared according to a protocol first reported by Warren and co-workers.[51] Given that the  $HBA$  and  $HPMA$  repeat units are isomeric and the same target DP was targeted for each (meth)acrylic block, these two diblock copolymers are structural isomers. However, a remarkable difference in their temperature-dependent behavior was observed on heating: the  $PHBA$  signals in the former copolymer became significantly more hydrated, whereas the  $PHPMA$  signals in the latter copolymer became slightly less hydrated (see Fig. 14). These observations are consistent with the *uniform* plasticization and *surface* plasticization mechanisms invoked to account for the observed morphological transitions, as discussed above. Interestingly, the apparent partial degree of hydration of the  $PHBA$  chains was as high as 80 %, although precise values depended on the particular proton signal selected for quantification (e.g. pendent (oxy)methylene vs acrylic backbone protons).

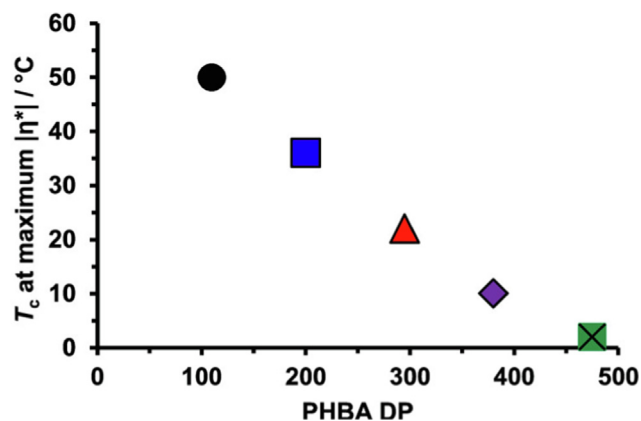
Recently, Cumming et al. prepared a series of  $PGMA_n$ - $PHBA_m$  diblock copolymers at pH 3 (see Fig. 15).[59] In this case, a relatively short water-soluble  $PGMA$  precursor (bearing no terminal carboxylic acid group) was chain-extended via RAFT solution polymerization of  $GMA$  prior to the RAFT aqueous dispersion polymerization of  $HBA$ . This approach enabled the  $PGMA$  DP to be conveniently varied from 57 to 300, while the corresponding  $PHBA$  DP was adjusted from 100 to 1580. When using a relatively long  $PGMA_{300}$  block, only kinetically-trapped spheres could be obtained. On the other hand, thermoresponsive diblock copolymer nano-objects were obtained for  $PGMA_{57-130}$ - $PHBA_{100-600}$ . This is in striking contrast to the analogous  $PGMA$ - $PHPMA$  system, for which



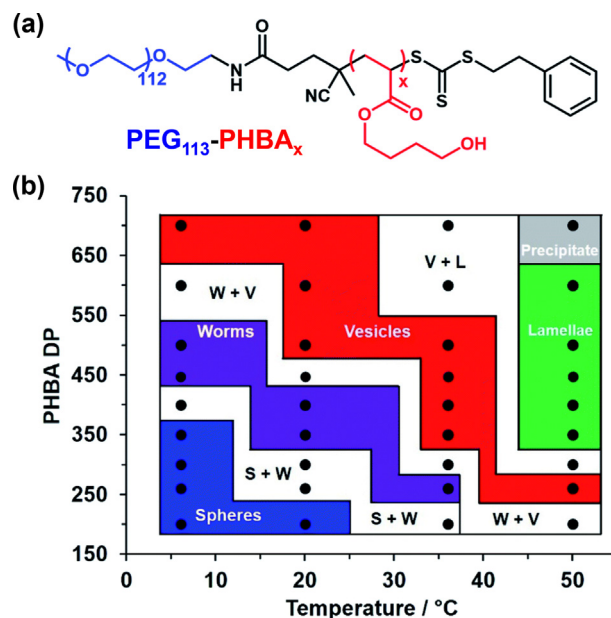


**Fig. 11.** (a) Chemical structure of a PNAEP<sub>85</sub>-PHBA<sub>295</sub> diblock copolymer. (b) Variation in z-average diameter (blue circles) and complex viscosity (red squares) with temperature for an aqueous dispersion of linear PNAEP<sub>85</sub>-PHBA<sub>295</sub> nano-objects. DLS studies were conducted on 0.1% w/w aqueous dispersions, while rheological measurements were performed on a 10% w/w aqueous dispersion for a run starting at 4 °C. (c) Prior to TEM analysis, 5% w/w aqueous dispersions of PNAEP<sub>85</sub>-PHBA<sub>295</sub> nano-objects were crosslinked with glutaraldehyde for 24 h at (i) 11 °C, (ii) 23 °C, (iii) 34 °C or (iv) 41 °C. (d) SAXS patterns recorded for a 1.0% w/w aqueous dispersion of linear thermoresponsive PNAEP<sub>85</sub>-PHBA<sub>295</sub> nano-objects at 5 °C (black data), 23 °C (blue data), 34 °C (purple data) and 41 °C (red data).<sup>[57]</sup> (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

the upper limit PHBA DP for thermoresponsive behavior is significantly lower.<sup>[48,82]</sup> Construction of a pseudo-phase diagram enabled identification of a range of diblock copolymer compositions corresponding to the pure worm phase at 20 °C. This was achieved by fixing the PHBA/PGMA molar ratio at approximately 2.1–2.5. The mean cross-sectional diameter of PGMA<sub>130</sub>-PHBA<sub>270</sub> worms was estimated to be 57 nm by TEM analysis (see Fig. 15c), which is significantly greater than that reported for thermoresponsive PGMA<sub>54</sub>-PHBA<sub>140</sub> worms.<sup>[82]</sup> As expected, thicker



**Fig. 12.** Relationship between the critical temperature ( $T_c$ ) corresponding to the maximum solution viscosity ( $|\eta^*|$ ) and the PHBA DP observed for five aqueous dispersions of PNAEP<sub>85</sub>-PHBA<sub>x</sub> nano-objects (where  $x = 110$  to 475).<sup>[57]</sup>

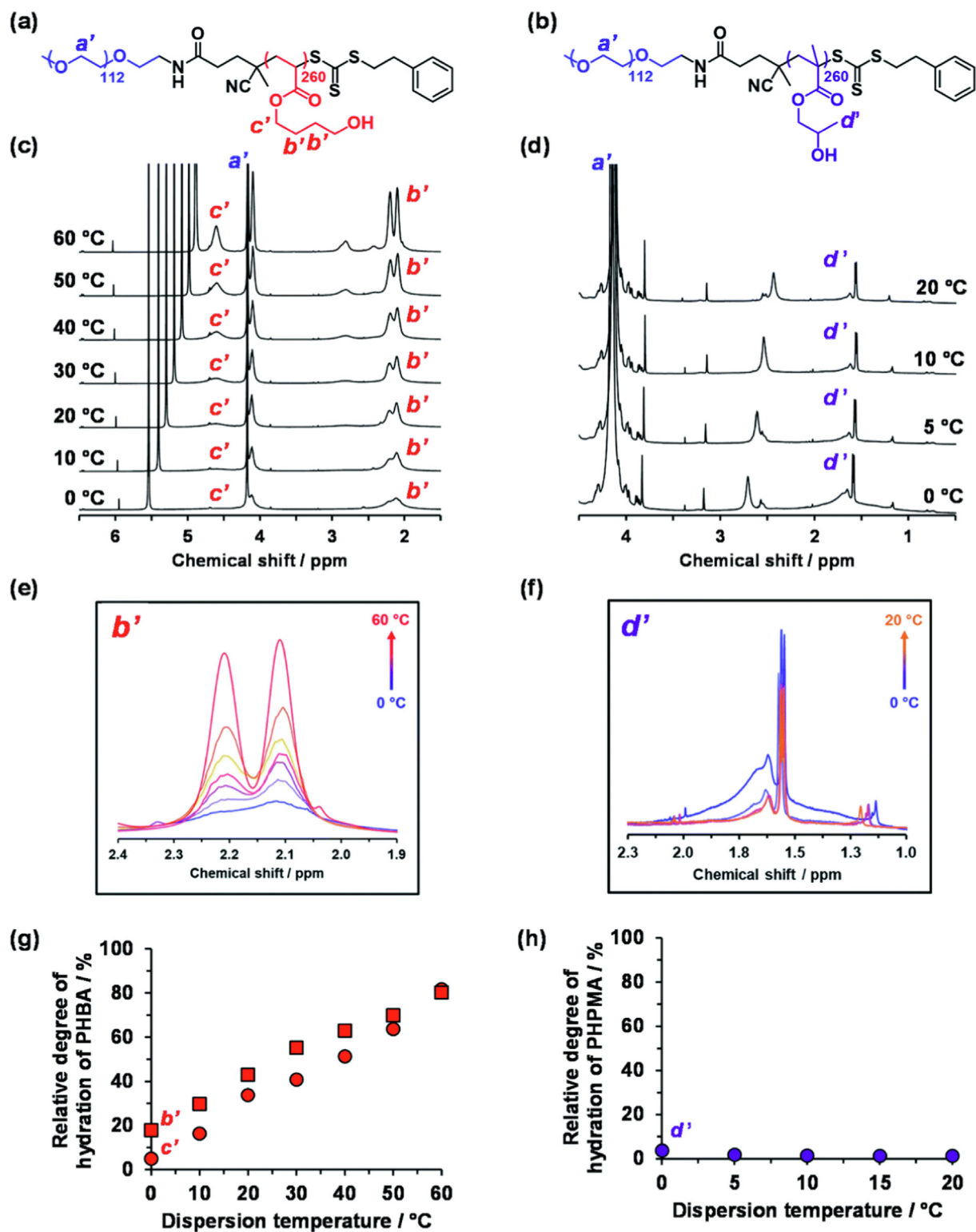


**Fig. 13.** Variable temperature phase diagram constructed for a series of linear PEG<sub>113</sub>-PHBA<sub>x</sub> nano-objects prepared via RAFT aqueous dispersion polymerization of HBA targeting 15% w/w solids. (N.B. S + W, W + V and V + L denote mixed phases comprising spheres plus worms, worms plus vesicles or vesicles plus lamellae, respectively).<sup>[58]</sup>

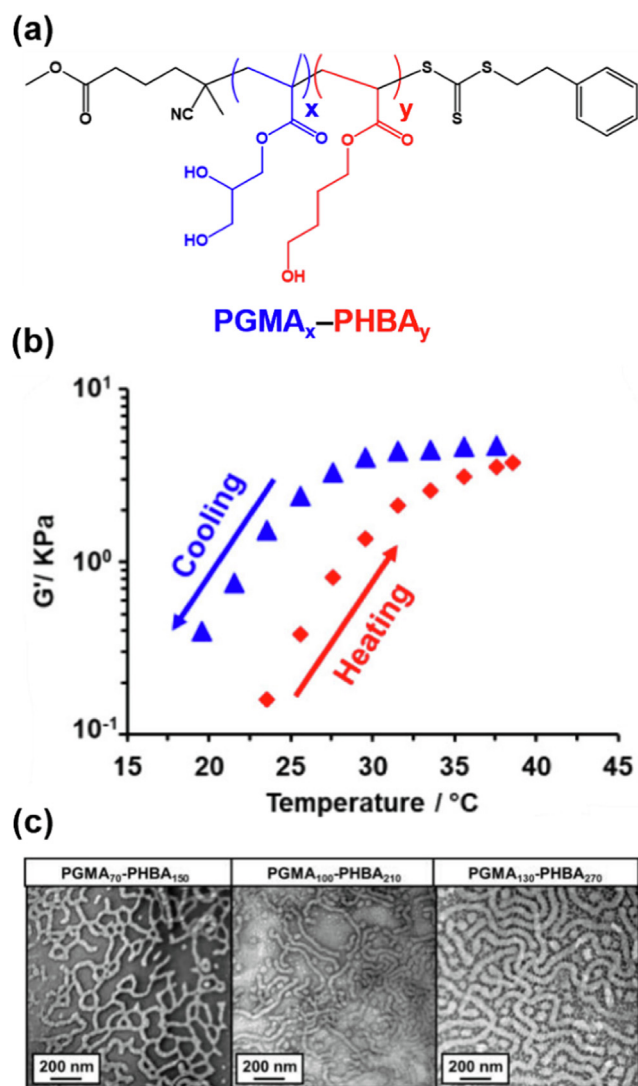
worms exhibit a lower critical gelation concentration, which is consistent with the 3D percolating network model proposed by Lovett and co-workers for such worm gels.<sup>[81]</sup>

In principle, such PGMA<sub>n</sub>-PHBA<sub>m</sub> worms should be useful for various cell biology applications.<sup>[93,95,113,114]</sup> For example, the diblock copolymer composition could be tuned to ensure that the critical temperature required for the formation of linear worms (and hence soft free-standing gels) is just below 37 °C, which would minimise the thermal shock experienced by cells during harvesting. Moreover, if the choice of a specific cell culture medium unduly influenced this critical temperature then the diblock copolymer composition could be further optimized to compensate for such an effect.

In a related study, Beattie and co-workers prepared a series of PHBA-based diblock copolymers using a water-soluble poly(2-hydroxyethyl acrylate) (PHEA) precursor.<sup>[60]</sup> However, this weakly hydrophilic steric stabilizer barely conferred colloidal stability on



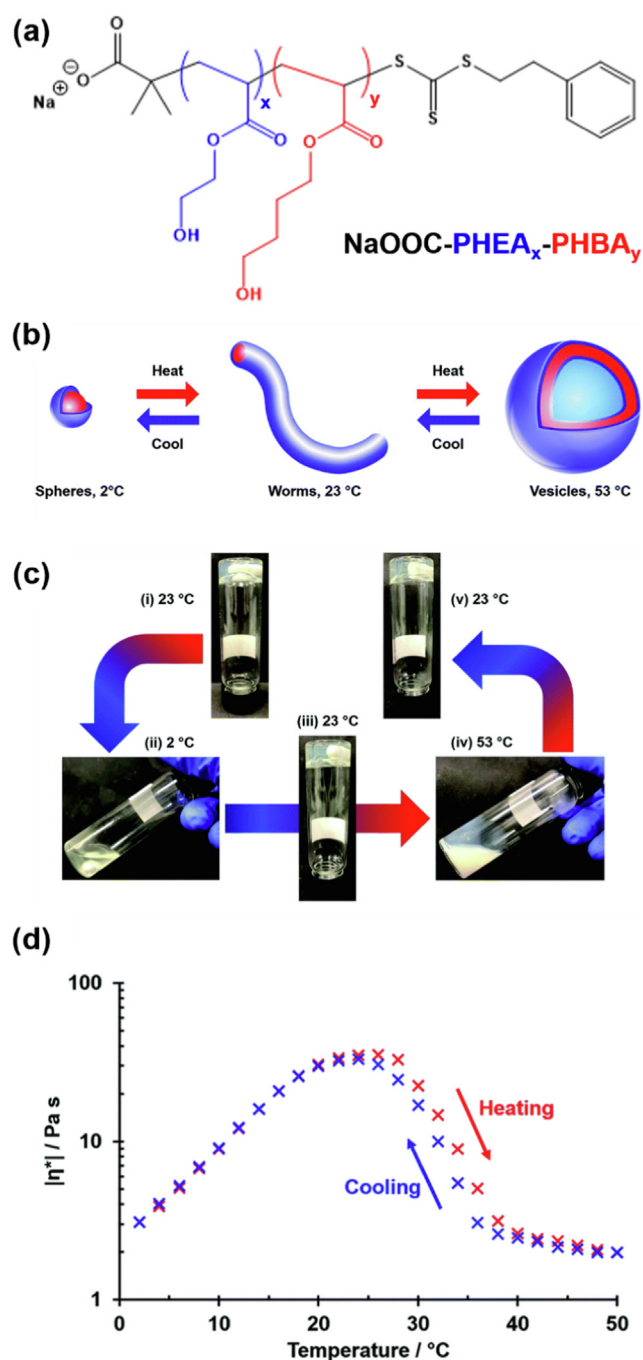
**Fig. 14.** Variable temperature <sup>1</sup>H NMR studies recorded for thermoresponsive linear PEG<sub>113</sub>-stabilized nano-objects dispersed in D<sub>2</sub>O. Chemical structures of (a) PEG<sub>113</sub>-PHBA<sub>260</sub> and (b) PEG<sub>113</sub>-PHPMA<sub>260</sub> diblock copolymers with assignment of the corresponding proton signals. (b) Normalized [relative to an external standard (pyridine in 1,1,2,2-tetrachloroethane-d<sub>2</sub>) at 7.2 and 6.0 ppm, respectively] partial <sup>1</sup>H NMR spectra recorded for a 15 % w/w aqueous dispersion of (c) PEG<sub>113</sub>-PHBA<sub>260</sub> and (d) PEG<sub>113</sub>-PHPMA<sub>260</sub> nano-objects prepared in D<sub>2</sub>O on heating from 0 °C to 60 °C. (e) Overlaid partial spectra recorded between 0 °C (blue data) and 60 °C (red data) for the four ethyl protons (b') at 1.9–2.4 ppm within the HBA repeat units. Clearly, the PHBA block becomes more hydrated at higher temperature. (f) Overlaid partial spectra recorded between 0 °C (blue data) and 20 °C (orange data) for the pendent methyl protons (d') at 1.3–2.0 ppm within the HPMA repeat units. The PHPMA<sub>260</sub> block is only weakly hydrated at 0 °C and becomes slightly dehydrated at higher temperature. Relative degrees of hydration calculated for the hydrophobic (g) PHBA<sub>260</sub> and (h) PHPMA<sub>260</sub> block as a function of temperature when using proton signals a', b', c' and d'. [58] (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).



**Fig. 15.** (a) Generic chemical structure of a  $\text{PGMA}_x\text{-PHBA}_y$  diblock copolymer. (b) Change in complex viscosity with temperature of a 10 % w/w aqueous dispersion of linear  $\text{PGMA}_{100}\text{-PHBA}_{325}$  nano-objects (heating run = red data; cooling run = blue data). (c) Representative TEM images recorded for  $\text{PGMA}_{70}\text{-PHBA}_{150}$  (number-average worm diameter = 32 nm),  $\text{PGMA}_{100}\text{-PHBA}_{210}$  (number-average worm diameter = 36 nm), and  $\text{PGMA}_{130}\text{-PHBA}_{270}$  (number-average worm diameter = 57 nm) worms crosslinked at 37 °C using glutaraldehyde.[59] (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

the nano-objects: end-group ionization of a terminal carboxylic acid group at pH 7 was essential to prevent their aggregation. Moreover, these linear nano-objects also proved to be unstable to dilution, which complicated their analysis. Covalent stabilization was attempted using glutaraldehyde but with mixed results. Nevertheless, the experimental data suggested that spheres, worms and vesicles could be formed under appropriate conditions (see Fig. 16). Overall, this PHEA-PHBA system proved to be rather more problematic than the prior formulations discussed above: this study highlights the importance of identifying a sufficiently hydrophilic steric stabilizer when targeting such shape-shifting thermoresponsive diblock copolymers.

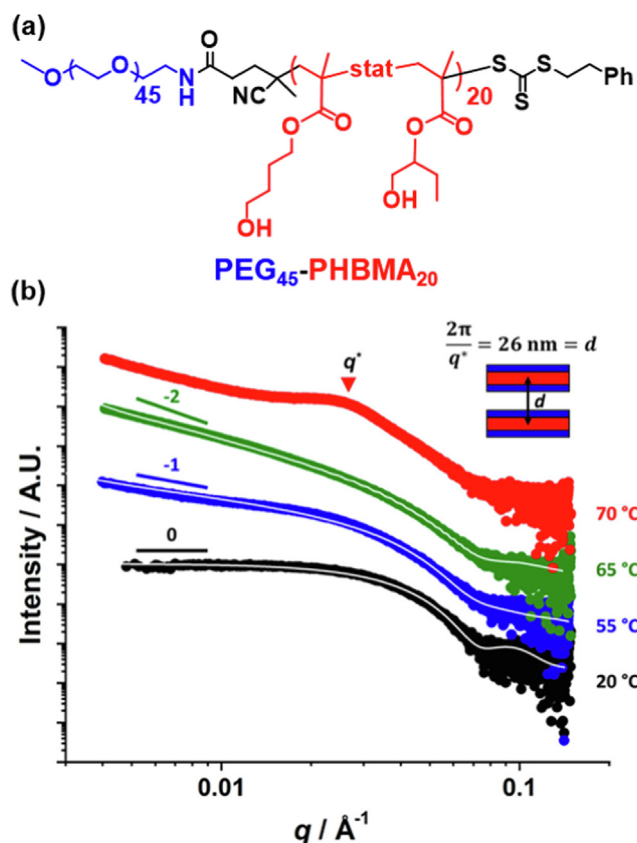
Perusing the PISA literature, it is clear that diblock copolymer nano-objects that exhibit thermally-induced morphological transitions are invariably prepared by RAFT dispersion polymerization. [47,51,115–118] This is because an essential prerequisite for such formulations is that the monomer that forms the structure-



**Fig. 16.** (a) Generic chemical structure of a  $\text{PHEA}_x\text{-PHBA}_y$  diblock copolymer and (b) schematic representation of its thermoreversible morphological transitions. Digital photographs recorded for a 20 % w/w aqueous dispersion of  $\text{PHEA}_{73}\text{-PHBA}_{265}$  nano-objects at pH 7, with 10 min equilibration being allowed at each temperature: (i) 23 °C; (ii) 2 °C; (iii) 23 °C; (iv) 53 °C; (v) 23 °C. (d) Variation in complex viscosity ( $|\eta^*|$ ) with temperature for the same 20 % w/w aqueous dispersion of  $\text{PHEA}_{73}\text{-PHBA}_{265}$  nano-objects at pH 7.[60]

directing block should be soluble in the chosen solvent. For example, a *water-miscible* monomer is required in the case of aqueous dispersion polymerization. This invariably means that the resulting water-insoluble block is only weakly hydrophobic, at least when compared to the analogous water-insoluble blocks prepared from *water-immiscible* monomers such as styrene or methyl methacrylate. Hence it is understandable that PISA formulations based on RAFT aqueous emulsion polymerization generally do not produce thermoresponsive diblock copolymer nano-objects.[62,79,80,86–8





**Fig. 17.** (a) Chemical structure of the PEG<sub>45</sub>-PHBMA<sub>20</sub> diblock copolymer. (b) Double logarithmic  $I(q)$  vs  $q$  SAXS patterns recorded for a 1.0 % w/w aqueous dispersion of thermoresponsive PEG<sub>45</sub>-PHBMA<sub>20</sub> nano-objects at 20 °C (black), 55 °C (blue), 65 °C (green) and 70 °C (red). The characteristic low  $q$  gradients expected for spheres, worms, and vesicles are 0,  $-1$  and  $-2$ , respectively. The mean distance,  $d$ , between stacked lamellae was calculated from the structure peak labeled  $q^*$  using the equation shown in the inset.[64] (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

8,119–126] However, we have recently reported an interesting counter-example, which is again based on a hydroxyl-functional vinyl monomer.[64] The monomer in question is hydroxybutyl methacrylate (HBMA), which comprises a 50:50 mixture of 2- and 4- isomers and has an aqueous solubility of approximately 25 g dm<sup>-3</sup> at 50 °C. A trithiocarbonate-capped PEG<sub>45</sub> precursor was used to polymerize HBMA when targeting a relatively short PHBMA DP of 20. According to SAXS studies, the resulting PEG<sub>45</sub>-PHBMA<sub>20</sub> formed spheres at 25 °C, worms at 58 °C, vesicles at

65 °C, and lamellae at around 70 °C, see Fig. 17b. Remarkably good reversibility was observed for each thermal transition, as judged by rheology, SAXS and DLS studies. In retrospect, this is no doubt owing to the relatively short PHBMA DP, which leads to a relatively low  $T_g$  for this methacrylic block. Indeed, in unpublished work we found that targeting PEG<sub>45</sub>-PHBMA<sub>50</sub> led to a substantial reduction in thermoresponsive behavior. A summary of each class of shape-shifting thermoresponsive diblock copolymer discussed in this Feature Article is provided in Table 1.

## 2. Conclusions

Shape-shifting thermoresponsive diblock copolymer nano-objects represent a fascinating new class of materials that exhibit unique self-assembly behavior in aqueous solution. Switching from PHPMA to PHBA offers a decisive advantage in terms of the reversibility of the thermally-induced morphological transitions owing to the much greater chain mobility of the latter acrylic polymer. Variable temperature NMR studies of PEG<sub>113</sub>-PHBA<sub>260</sub> and PEG<sub>113</sub>-PHPMA<sub>260</sub> nano-objects indicate that the relative degree of (partial) hydration has a weak negative temperature dependence for the PHPMA block but a strong positive temperature dependence for the PHBA block. This is highly counter-intuitive given that PHPMA and PHBA are structural isomers.

Amphiphilic PHBA-based diblock copolymers can be redispersed from the solid state directly into cold aqueous solution to produce spheres, worms, vesicles or lamellae. The linear worm phase is readily identified by the maximum solution viscosity observed for a viscosity vs temperature plot. If desired, the critical temperature corresponding to the formation of linear worms can be adjusted over a wide range simply by varying the diblock copolymer composition. Thus, targeting a longer PHBA DP when employing a fixed steric stabilizer DP leads to a higher critical temperature being required to produce pure worms. Unlike the corresponding amphiphilic PHPMA-based diblock copolymers, targeting a relatively long PHBA DP does not result in any loss of thermoresponsive behavior. Moreover, this enables extremely thick worms to be targeted with a mean cross-sectional diameter exceeding 100 nm.

Initially, shape-shifting thermoresponsive behavior was only observed at low pH because this was required to suppress ionization of the terminal carboxylic acid group originating from the RAFT agent. However, subsequent formulations involving either non-ionic PEG or PGMA stabilizer blocks containing no ionizable end-groups confirmed that the same shape-shifting behavior also occurred at neutral pH, which suggests potential bio-applications.

Given the optimized loading efficiency for the encapsulation of silica nanoparticles within vesicles reported by Czajka and co-workers,[111] such shape-shifting thermoresponsive diblock

**Table 1**

Summary of the various literature examples of shape-shifting thermoresponsive block copolymer nano-objects prepared via polymerization-induced self-assembly (PISA).

diblock copolymer composition	nature of thermoresponsive behavior	copolymer morphologies	reversibility	crosslinking chemistry	Ref.
PGMA <sub>54</sub> -PHPMA <sub>140</sub>	Surface plasticization	S (5 °C), W (25 °C)	excellent	none	[47]
PEG <sub>113</sub> -PHPMA <sub>300</sub>	Surface plasticization	S (2 °C), V (25 °C)	poor	none	[51]
PMPC-PPMA	Surface plasticization	S (6 °C), V (25 °C)	poor	none	[101]
PHPMA <sub>41</sub> -PHPMA <sub>180</sub>	Surface plasticization	S (4 °C), W (22 °C), V (50 °C)	poor	none	[55]
PDMAC <sub>56</sub> -P(HBA- <i>stat</i> -DAAM) <sub>264</sub>	Uniform plasticization	S (1 °C), W (25 °C), V (50 °C), L (50 °C)	good	ADH	[56]
PDMAC <sub>49</sub> -P(HBA- <i>stat</i> -DAAM) <sub>302</sub>	Uniform plasticization	S (5 °C), V (50 °C)	good	DS-ADH	[111]
PNAEP <sub>85</sub> -PHBA <sub>295</sub>	Uniform plasticization	S (11 °C), W (23 °C), V (35 °C), L (41 °C)	excellent	glutaraldehyde	[57]
PEG <sub>113</sub> -PHBA <sub>350</sub>	Uniform plasticization	S (6 °C), W (20 °C), V (36 °C), L (50 °C)	excellent	glutaraldehyde	[58]
PGMA <sub>100</sub> -PHBA <sub>325</sub>	Uniform plasticization	S (2 °C), W (10 °C), V (30 °C)	excellent	glutaraldehyde	[59]
PHEA <sub>73</sub> -PHBA <sub>265</sub>	Uniform plasticization	S (2 °C), W (23 °C), V (53 °C)	excellent	glutaraldehyde	[60]
PEG <sub>45</sub> -PHBMA <sub>20</sub>	Uniform plasticization	S (25 °C), W (58 °C), V (65 °C), L (75 °C)	excellent	none	[64]

N.B. S, W, V and L denote spheres, worms, vesicles and lamellae, respectively.



copolymers may prove to be useful for microencapsulation applications, particularly for formulations that require triggered release. Moreover, the relatively mild encapsulation conditions should be beneficial when attempting to load such vesicles with enzymes or other delicate cargoes. However, the development of new reversible crosslinking chemistries would be extremely useful for further advances to be made in this particular sub-field.

Finally, the first example of a shape-shifting thermoresponsive diblock copolymer via RAFT aqueous emulsion polymerization has been achieved by employing hydroxybutyl methacrylate (HBMA). The unexpected success of this latter formulation can be attributed to two important factors: (i) the relatively high aqueous solubility of this methacrylic monomer ( $25 \text{ g dm}^{-3}$  at  $50 \text{ }^\circ\text{C}$ ) and (ii) the relatively low degree of polymerization of 20 targeted for the weakly hydrophobic PHBA block (which leads to a correspondingly low  $T_g$  and hence relatively high chain mobility).

### 3. Prospect

In principle, the efficient encapsulation of one or more enzymes within PHBA-based vesicles should offer new opportunities to study cascade reactions [127,128] by taking advantage of the highly porous nature of the partially hydrated PHBA membrane. However, this application would most likely require the development of new reversible crosslinking chemistry to stabilize the enzyme-loaded vesicles and hence prevent morphological transitions during their handling and long-term storage.

The Flory-Huggins parameter for the PHBA-water interaction should be determined by conducting hydration studies on PHBA thin films using ellipsometry.[129] This vital information should underpin new mean field theory studies, which are expected to shed further light on the thermoresponsive behavior of such amphiphilic diblock copolymers.

We hypothesize that labeling PHBA-based chains with appropriate fluorophores should enable the first observation of diblock copolymer morphological transitions using super-resolution microscopy. In this context, targeting relatively high PHBA DPs should produce relatively thick worms. In principle, this could facilitate imaging of the surface of a worm gel for the direct visualization of the postulated 3D percolating network formed by the weakly interacting worms.[81]

Our time-resolved SAXS studies of various PISA formulations [130–133] could be extended to include the RAFT aqueous dispersion polymerization of HBA. This would enable the evolution in copolymer morphology to be examined during such syntheses when targeting the lamellar phase. Finally, we have recently reported the development of so-called *reverse sequence* PISA formulations based on the synthesis of charge-stabilized PHPMA latex particles.[134,135] It would be fascinating to evaluate the feasibility of performing the analogous reverse sequence PISA syntheses using HBA instead of HPMA to produce new examples of thermoresponsive diblock copolymer nano-objects. If successful, the organosulfur RAFT group would be located at the end of the hydrophilic steric stabilizer block rather than the weakly hydrophobic structure-directing block, which should aid its removal.

### Data availability

Data will be made available on request.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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